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Rotterdam Advanced Multiple Plate: A novel method to measure cold hyperalgesia and allodynia in freely behaving rodents

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HIGHLIGHTS

- Spared nerve injury was used to induce cold hypersensitivity in rats.
- RAMP is the new objective method to measure cold hypersensitivity.
- RAMP is compared to the paw withdrawal method.
- Paw withdrawal method confirms that the SNI rats are cold hypersensitivity.
- RAMP shows comparable results as the paw withdrawal method.

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ABSTRACT

Background: To investigate the pathophysiology of temperature hypersensitivity in neuropathic pain rodent models, it is essential to be able to quantify the phenotype as objective as possible. Current temperature sensitivity measuring paradigms are performed during exposure to external factors, i.e. light, sound and smell, which modulate behavior significantly. In addition the present outcome measure for temperature hypersensitivity in rodents is the examination of the hind paw lift upon exposure to a certain temperature, which reflects more a reflex-flexion than an experience of pain.
New method: Therefore the Rotterdam Advanced Multiple Plate (RAMP) was developed to assess cold hyperalgesia and allodynia objectively in freely behaving neuropathic pain rats, which measures the avoidance for certain temperatures and monitoring the location of the rat with an infrared camera while excluding external environmental influences such as light and sound.
Results: Compared to sham rats, the spared nerve injury (SNI) rats demonstrated a higher preference for the comfortable plate (27 °C) when the other three plates were set at 5 °C, 14 °C, 17 °C and 19 °C. We were unable to detect heat hyperalgesia and allodynia with the RAMP.
Comparison with existing method: The paw withdrawal method displays similar results during cold hypersensitivity measurements as observed with the RAMP. The SNI group did display heat hypersensitivity during the paw withdrawal test.
Conclusions: The results indicate that the RAMP is able to quantify cold hyperalgesia and allodynia in neuropathic pain rats while resolves some of the problems of conventional temperature sensitivity measuring paradigms in rodents.

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1. Introduction

Neuropathic pain is a debilitating disease with a wide range of symptoms, including spontaneous pain and hypersensitivity to mechanical and temperature stimuli (Baron, 2006). The temperature hypersensitivity, includes heat or cold hyperalgesia (an increased sensitivity to a normally painful heat or cold stimuli) and heat or cold allodynia (pain due to a heat or cold stimulus that does not normally provoke pain) (Coutaux et al., 2005).

With the identification of the thermo-sensitive ion channels, the transient receptor potential (TRP) channels, it is now possible to investigate the pathophysiology of temperature hypersensitivity in neuropathic pain animal models (Caterina, 2007). However, despite the identification of these TRP channels, the pathophysiology of temperature hypersensitivity after peripheral nerve injury remains elusive (Fleetwood-Walker et al., 2007; Katsura et al., 2006; Xing et al., 2007), which partly may be attributed to the difficulty in determining temperature hypersensitivity objectively in rodents.

The current method to examine heat or cold hypersensitivity is to measure the time between the first contact of the hind paw with a heat or cold plate and the first paw withdrawal, the so-called 'paw lift' latency (Jasmin et al., 1998). This 'paw lift' technique, however, is not always performed or scored similarly. For example, some reports measure the total number of 'paw lifts' (Yalcin et al., 2009), while others measure the cumulative 'paw lifts' (Tanimoto-Mori et al., 2008) or the latency to the first 'paw lift' (Allchorne et al., 2005; Tanimoto-Mori et al., 2008).

The Rotterdam Advanced Multiple Plate (RAMP) is a novel device that measures the preference of the animals to avoid plates with specific temperatures and is therefore an alternative to measuring paw lifts. The RAMP consists of four plates, with always one plate at a comfortable temperature. The temperatures of the other three plates are systematically manipulated during the experiment; subsequently, the preference of the animal for the comfortable plate is used as a measure of temperature hypersensitivity. Thus, the philosophy of the RAMP is that neuropathic pain rats will have a higher preference for the comfortable plate as compared to control rats, avoiding the other colder or hotter plates. By design, the RAMP has a number of theoretical advantages over the paw lift method. Since the animal is free to move, it can avoid painful stimuli. In addition, reflex action does not play a role as it may during paw stimulation in the paw lift technique (Borszcz et al., 1992). Furthermore, since neuropathic pain animal models do not only develop temperature hypersensitivity but also spontaneous pain (Wang and Wang, 2003) and hypersensitivity to mechanical stimuli (Mogil, 2009; Mogil et al., 2010), it is uncertain if a 'paw lift' is only caused by temperature hypersensitivity or is also due to the spontaneous pain that might be enhanced during the test. Besides these confounders, there may be intra-individual differences in scoring the paw lift because of the subjective nature of scoring the paw lifts.

In the current study, we used the spared nerve injury (SNI) model (Decosterd and Woolf, 2000), which is a well-established neuropathic pain animal model that is known to develop long-term cold and heat hypersensitivity. The hypothesis of the current experiments was that the SNI group would have a higher preference for the comfortable plate as compared to the sham group, indicating that the RAMP can objectively measure hyperalgesia and allodynia for cold and heat in the SNI rat model.

2. Methods

All experiments were approved by the Dutch Ethical Committee on Animal Welfare (DEC) and all procedures adhered to the European guidelines for the care and use of laboratory animals (Council Directive 86/6009/EEC). We used 35 Male Wistar rats weighing 250 g. These were divided into an SNI ($n = 20$) and a sham group ($n = 15$). The same SNI rats were used in the experiments and were also followed over time. The control group presented is the one-week postoperative sham group. Two rats per cage were housed with free access to water and food and maintained on a 12 h dark-light cycle.

2.1. SNI-surgery

Under isoflurane (2%) anesthesia the skin on the left lateral surface of the thigh was incised and the biceps femoris muscle was divided and spread lengthwise to expose the three branches of the sciatic nerve: i.e. the sural, common peroneal and tibial nerves. Two of the three terminal branches, the common peroneal and the tibial nerves were tight ligated and subsequently transected with 5.0 silk sutures. Great care was taken to avoid any contact with or stretching of the intact sural nerve. For sham controls we performed the same surgery without the ligatures and lesions, leaving the nerves intact. The skin was sutured and the animals were allowed to recover. In all cases, postoperative analgesia was provided by subcutaneous administration of buprenorphine (0.05–0.1 mg/kg; Temgesic; Schering-Plough BV, Utrecht, the Netherlands). Animals were monitored daily for signs of stress or discomfort but in all cases recovered uneventfully with no autotomy.

2.2. Paw lift thermal test

As a reference for the RAMP measurements, we used the conventional paw lift method with the hot and cold plate test device. Before testing, all animals were habituated for 5 days by the same person placing the animals in the box during 10 min, with the plates at room temperature. The device consisted of an aluminum plate (21 cm × 21 cm of surface and 2 cm thick) coiled with PVC tubes that circulated water with 50% methanol diluted and a see-through Plexiglas around it. The rat was placed on the hot (37 °C, 40 °C, 43 °C and 50 °C) or cold plate (5 °C, 14 °C, 17 °C and 19 °C). A paw lift was taken as a positive response, which is defined as a quick flutter or flinch of the affected hind paw. The time difference between the start of the paw contact with the plate until a positive response was noted, was called the latency time. A maximum cut off time of 150 s was used to prevent tissue damage at the lower and higher temperatures. Each rat was only tested once on any given test day to avoid any possible anesthetic or tissue damage effects that could be produced by repeated exposure to a cold or hot surface. The tester performing the paw withdrawal test was blind to the treatment. In the first week postoperatively we examined the animals daily with the paw lift thermal test. However, because we did not observe evidential differences between the sham and SNI group in the first week, the decision was made to take 1 week as the first time-point post-surgery.

2.3. The Rotterdam Advanced Temperature Multiple Plate (RAMP)

The Rotterdam Advanced Multiple Plate consists of 4 aluminum plates, each 21 cm × 21 cm × 2 cm ($L \times W \times H$) (Fig. 1). The plates contain fluid channels, which are connected to water bath A and water bath B (both Haake K20) through polyvinyl chloride (PVC) tubes and 8 valves (three way Solenoid valve-dry 3/2 – NC). Each water bath is set at a different electronically controlled temperature (for example: water bath A cold, water bath B warm) in the range from 0 to 50 °C (with an accuracy of ±1 °C). Distilled water is continuously pumped through the system at a maximum flow rate of 12.5 L/min and at a maximum pressure of 300 mbar. The valves are controlled by a computer program developed in LabVIEW 8.5 (National Instruments) via a NI-6008 I/O hardware card and solid-state relays to provide power to the valves. A pair of valves for each